

reported human UCP-2 cDNA [Gimeno, R. et al. (1997), Diabetes, Vol. 46, 900-906] and the base sequence complementary to base number 1300 to 1329: 5'-ATACAGGCCGATGCGGACAGAGGCAAAGCT-3' as oligonucleotide primers, human UCP-2 gene was amplified by PCR (after heating at 94°C for 5 min., a cycle consisting of heating at 94°C for 1 min, 55°C for 0.5 min and 72°C for 1.5 min was repeated 30 times, followed by heating at 72°C for 5 min), then inserted into pCR-blunt vector.

Using this plasmid DNA carrying the insert as the template, oligonucleotide primers were prepared, and probes were prepared using PCR DIG probe synthesis kit (Boehringer-Mannheim Co.) following the attached instruction. Using the prepared probes, human genomic DNA library (Clontech Laboratory, California, USA) in 3×10^6 phages was screened using nitrocellulose filters. Plaque hybridization was performed using DIG Easy hyb (Boehringer-Mannheim Co.), DIG Wash and Block Buffer Set (Boehringer-Mannheim Co.), and DIG nucleic acid detection kit (Boehringer-Mannheim Co.) following the attached instruction. As a result, eight positive clones were obtained from 3×10^6 phages. Of these clones, an inner primer of non-coding exon of previously reported human UCP-2 cDNA sequence [Gimeno, R.E. et al. (1998), Diabetes, 47 (4), 685-687] was synthesized (5'-CAAAGCTGCCAGTGGCTATCATGGCCCG-3'), and a clone containing the non-coding exon was detected by PCR using a primer containing EMBL3 sequence (5'-GACCGGTCGACCCAGATCTGGGTCGACCTG-3'), and a genomic clone containing the 5' upstream region of UCP-2 was obtained. From the genomic clone, 3.5 kbp fragment containing UCP-2 promoter region was prepared, and inserted into pCR-blunt vector (Invitrogen Co.), and transformant *E. coli* TOP/10 pCR-UCP2P5' #1-10 was prepared. After that, the restriction enzyme map was prepared and the base

sequence was determined. The determined base sequence is shown in Figures 1 to 6. As shown in Figures 1 to 6, base number 2271 - 2326 and 3416 - 3505 were completely consistent with human UCP-2 cDNA (Gimeno, R.E. et al. (1998), Diabetes, 47 (4), 685-687). Furthermore, the terminal base sequences of the consistent regions were consistent with Shahnborn rule, which is the characteristic of intron-exon boundary base sequence, suggesting that the consistent base sequences are introns. A sequence likely to be CpG island (base number about 2070 - 2270), which is a characteristic of promoters without containing TATA-box sequence, was also confirmed upstream of the first exon. In the promoter sequence described above, PPRE (base number 284 - 296), which is the regulator sequence of promoters of fat cell-related genes, and three C/EBP binding sites (base number 1316 - 1320, 1364 - 1368, 1698 - 1692) were confirmed.

Example 2 Examination of human UCP-2 gene promoter activity

To confirm the promoter activity of the cloned genomic DNA fragment, luciferase assay was performed. pGL3-Basic plasmid (Promega Co.) carrying firefly luciferase gene as the reporter gene was used for the vector. As the internal standard, pRL-SV40 plasmid (Promega Co.) expressing sea pansy luciferase under control of SV40 promoter was used.

EcoRI fragment (3.5 kb) was isolated from the genomic human UCP-2 DNA and blunted using Blunting High Kit (TOYOBO Co.), and then ligated to SmaI-digested pGL3-Basic plasmid DNA. Following the above procedure, human UCP-2 promoter/luciferase vector (pGL-3UCP2) was constructed in which the base number 1 - 3505 shown in Figures 1 to 6 was inserted into pGL3-Basic vector.

The constructed human UCP-2 promoter/luciferase vector was transiently transfected in HepG2 cells, in which constant expression of UCP-2 was confirmed by RT-PCR, and the activity was examined.

5 HepG2 cells were seeded in 24-well multiplates (Nunk Co.) at a density of 60,000 cells/well, and cultured at 37°C in 5% CO₂ overnight. Using SuperFect Transfection Reagent (QIAGEN Co.), cells were transiently transfected with 1 µg of human UCP-2
10 promoter/luciferase vector DNA or pGL3-Basic DNA and 0.1 µg of pRL-SV40 DNA. The procedure was performed according to the attached instruction. Then, the cells were cultured at 37°C in 5% CO₂ for 24 hours, and the luciferase activity was detected using PicaGene Dual
15 Sea Pansy (Nippon Gene Co.) according to the attached instruction. The measurement data were presented as relative activity to the internal standard value of pRL-SV40-derived sea pansy luciferase activity. The results are shown in Figure 7. The human UCP-2
20 promoter/luciferase vector-derived luciferase activity was markedly higher than that of pGL3-Basic lacking the promoter. Therefore, the genomic DNA of human UCP-2 gene of this invention has a promoter activity reflecting the in vivo UCP-2 gene expression system.

25 Example 3 Examination of human UCP-2 gene promoter activity in human differentiated fat cell-like cells

Using the human UCP-2 promoter/luciferase vector DNA obtained in Example 2, the promoter activity in
30 human fat cell-like cells differentiated from MG-63 cells was confirmed.

MG-63 cells were seeded in 24-well multiplates (Nunk Co.) at a density of 100,000 cells/well, and cultured at 37°C in 5% CO₂ overnight. Using SuperFect
35 Transfection Reagent (QIAGEN Co.), cells were